

Supplemental Figure Legends

Supplemental Figure 1. ERCC1 foci formation is dependent on the presence of SLX4. (A) Indirect immunofluorescence with an antibody ERCC1 in U2OS cells transfected with a combination of three siRNAs against SLX4 or against Luciferase (Luc) as a control. Efficiency of SLX4 knockdown is shown in Figure 1B. The U2OS cells were pre-extracted with Triton-X100 before fixation. Nuclei were stained with DAPI. (B) Indirect immunofluorescence with an antibody against ERCC1 in BJ/hTERT, RA3083/hTERT and RA3331/E6E7/hTERT cell lines. The cell lines were prepared as above. (C) Indirect immunofluorescence staining of RA3331/E6E7/hTERT expressing indicated SLX4 mutants anti-ERCC1 and anti-HA antibodies. Nuclei were stained with DAPI. The cell lines were prepared as above.

Supplemental Figure 2. Analysis of interacting partners of SLX4 mutants listed in Figure 2A. (A) and (B) The cell extracts from RA3331/E6E7/hTERT cell lines expressing corresponding SLX4 mutants were subjected to immunoprecipitation using anti-HA antibody or control IgG. SLX4, XPF, ERCC1, and MUS81 were identified by immunoblotting with appropriate antibodies.

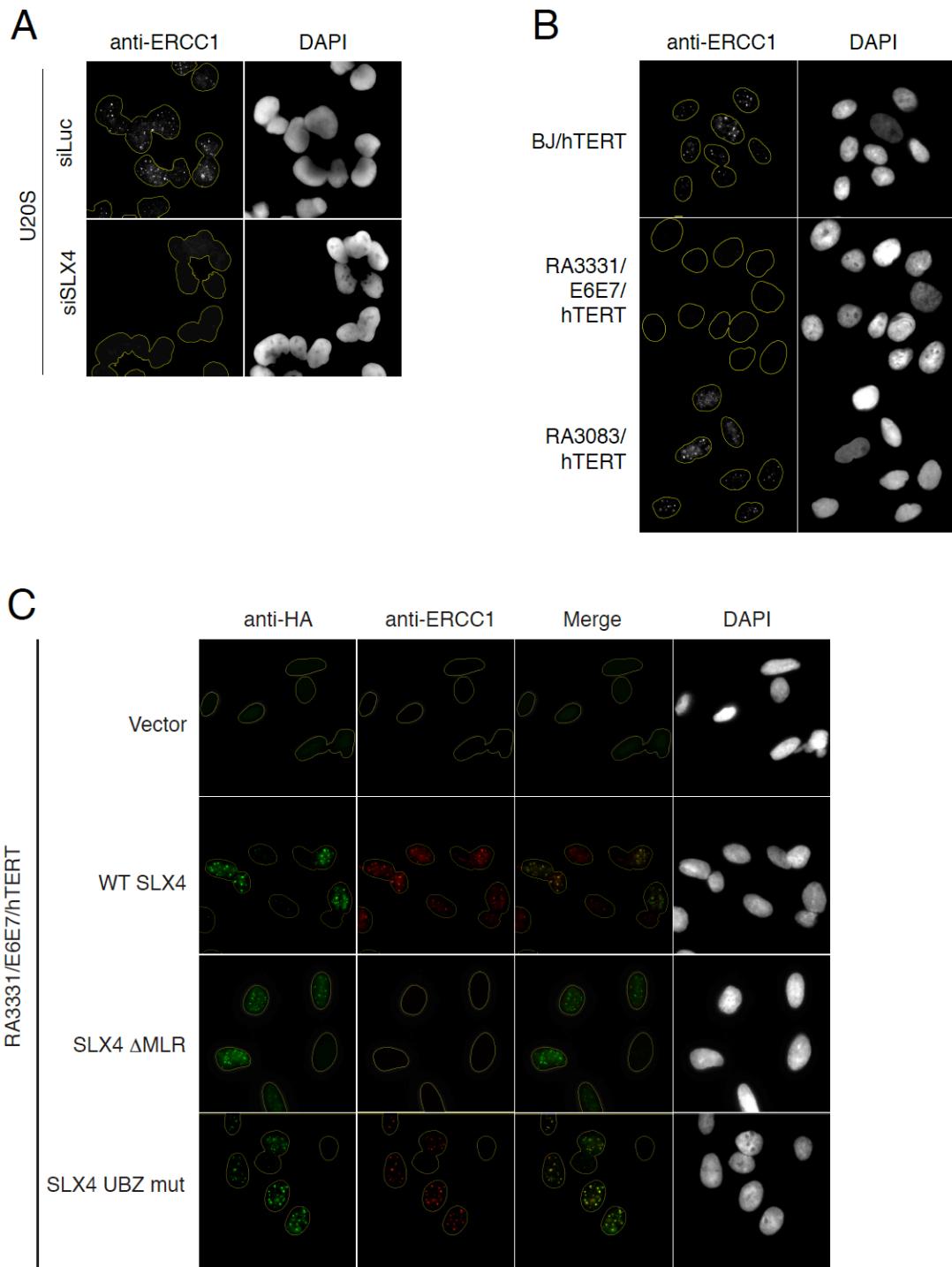
Supplemental Figure 3. Sensitivity assays of RA3331/E6E7/hTERT cell lines expressing indicated SLX4 mutants. (A) MMC sensitivity in RA3331/E6E7/hTERT cell lines expressing indicated SLX4 mutants (B) CPT sensitivity in RA3331/E6E7/hTERT cell lines expressing indicated SLX4 mutants (C) PARP inhibitor sensitivity assay of RA3331/E6E7/hTERT cell lines expressing indicated SLX4 mutants.

RA3331/E6E7/hTERT fibroblast cell lines expressing wild type SLX4, empty vector, and various SLX4 mutants listed in figure 1A were treated with different levels of MMC (0-100 nM), CPT (0-16 nM) and PARP inhibitor (0-10 μ M) in triplicate. After 8 days in culture, the cell number was determined with a Coulter counter. The number of cells at each concentration was divided by the number of cells in the untreated plate to calculate the percentage of cell survival. The error bars indicate standard deviations from three replicates.

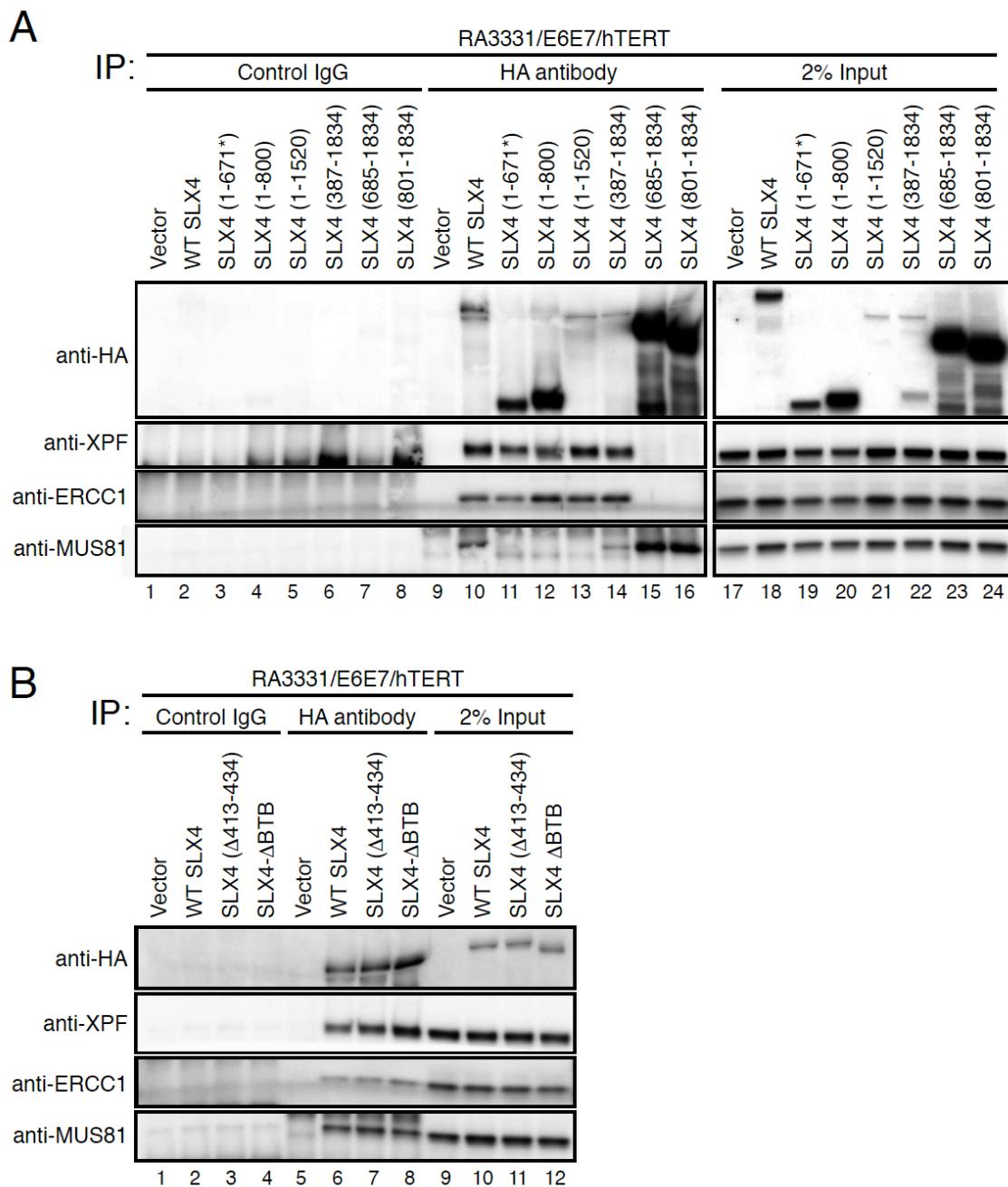
Supplemental Figure 4. MMC sensitivity assays of the indicated FA fibroblast cell lines.

All cell lines have been transduced with HPV E6E7. Indicated cell lines were treated in triplicate with increasing concentration of MMC (1-100 nM). After 7 days in culture, the cell survival was determined using the Cell Titer-Glo reagent. The error bars indicate standard deviations from three replicates.

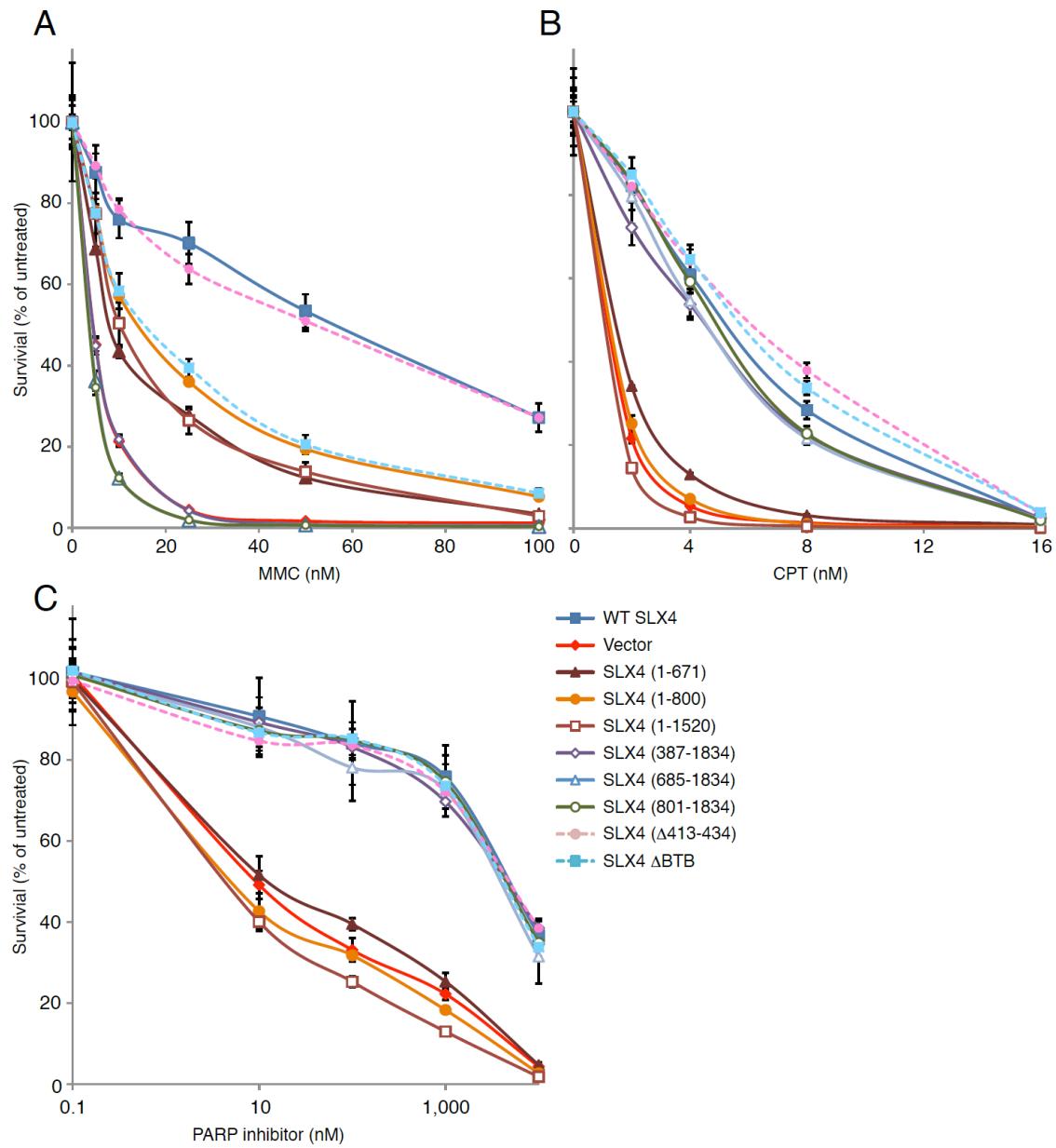
Supplemental Figure 1.



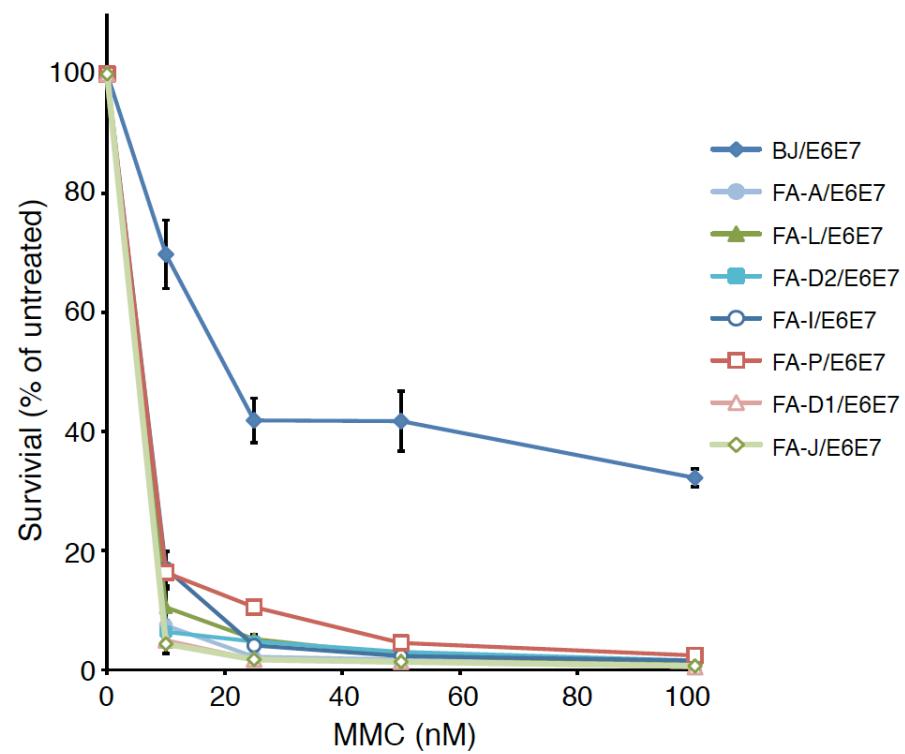
Supplemental Figure 2



Supplemental Figure 3.



Supplemental Figure 4.



Supplemental Table 1. FA cell lines used this study

RA	Mutant Gene	Mutation 1	Predicted Protein Effect 1	Mutation 2	Predicted Protein Effect 2	Cell line reference
3087	FANCA	c.793-?_4368+?del (delEx9_43)	del	c.793-?_4368+?del (delEx9_43)	del	this study
3100	FANCL	c.1007_1009delTAT	p.Ile336_Cys337del insSer	c.1095_1098dupAATT	p.Thr367AsnfsX13	(Ali et al., 2009)*
2645	FANCD2	c.2444G>A	p.R815Q	c.2715+1G>A	p.E906LfsX4	(Kalb et al., 2007)*
2480	FANCI	c.157+78G>A [in the cDNA: c.157_158ins85 (inserted intronic sequence: c.157+80_157+164 (exonification)]	p.G53VfsX2	c.3493delG	p.D1165TfsX24	this study
2374	FANCJ/ BRIP1	c.2392C>T	p.R798X	c.2392C>T	p.R798X	(Levran et al., 2005)*
3226	FANCD1/ BRCA2	c.658_659delGT	p.V220IfsX4	c.5946delT	p.S1982RfsX32	this study
3331	FANCP/ SLX4	c.514delC	p.Leu172PhefsX22	c.2013+225_3147 del4890insCC	p.Leu672ValfsX119	(Kim et al., 2011)*

***References for supplemental table 1**

Ali AM, Kirby M et al. Identification and characterization of mutations in FANCL gene: a second case of Fanconi anemia belonging to FA-L complementation group. *Hum Mutat* 2009; 30: E761-770.

Kalb R, Neveling K et al. Hypomorphic mutations in the gene encoding a key Fanconi anemia protein, FANCD2, sustain a significant group of FA-D2 patients with severe phenotype. *Am J Hum Genet* 2007; 80: 895-910.

Kim Y, Lach FP et al. Mutations of the SLX4 gene in Fanconi anemia. *Nat Genet* 2011; 43: 142-146.

Levran O, Attwooll C et al. The BRCA1-interacting helicase BRIP1 is deficient in Fanconi anemia. *Nat Genet* 2005; 37: 931-933.

Supplemental Table 2. Cloning primers

SLX4 (1-800)	GGGGACAAGTTGTACAAAAAAGCAGGCTTAATGAAACTGAGTGTGAATGAGG
	GGGGACCACTTGTACAAGAAAGCTGGGTCTCACCATGGTTGCCCTCTGAGT
SLX4 (1-1520)	GGGGACAAGTTGTACAAAAAAGCAGGCTTAATGAAACTGAGTGTGAATGAGG
	GGGGACCACTTGTACAAGAAAGCTGGGTCTCACCTCTGCTCTCCCCGTC
SLX4 (387-1834)	GGGGACAAGTTGTACAAAAAAGCAGGCTTAATGTTCAGCTCAGTGATCACA
	GGGGACCACTTGTACAAGAAAGCTGGGTCTCAGTCCGCTCACCTT
SLX4 (685-1834)	GGGGACAAGTTGTACAAAAAAGCAGGCTTAATGACTGCTCAAAGAAGAAGCG
	TAAGATGGTCAATAACCCACACCTGAGT
SLX4 (801-1834)	GGGGACAAGTTGTACAAAAAAGCAGGCTTAATGACTGCTCAAAGAAGAAGCG
	TAAGTGGGAGGAGAAGGAAGCAGAGA
SLX4-DSBD	GGGGACCACTTGTACAAGAAAGCTGGGTCTCAGTCCGCTCACCTT
	GGGGACAAGTTGTACAAAAAAGCAGGCTTAATGAAACTGAGTGTGAATGAGG
	GGGGACCACTTGTACAAGAAAGCTGGGTCTCACTGGGAGGCGAGGGTCTGG

Supplemental Table 3. Mutagenesis primers

SLX4_C296A_C299A	GGAAAAGGGTTTGTCTCGCCCAGATTGCTAAAAGAACCTCTCAGCC
SLX4_C336A_C339A	CCTCAGATCCCTGAGGCCCGATTGCTGGAAACCGTTCTTACC
SLX4-DMLR-Motif	GGAAGAGGCAGGCGAAGGGTGCAGCTGTACC
	GGTACAGCCGCACCCCTCCGCCTCTCC
SLX4DMLR	GCAAGAAGGAGCCACGGCCCCAGCGGCTGCC
	GGCAGGCCGCTGGGCCGTGGCTCCTTCTGC
SLX4DBTB	GCTGGTTGCTGACTTGGCGTGCCTATTGCCACTGACTC
	GAGTCAGTGGCAATAGGCACGCCAAAGTCAGCAACCAGC
SLX4DSAP	GGTGGAGCTCAGAAGCCCCCTCACTGCCAGACCC
	GAGGGTCTGGCAGTGAGGGGGCTCTGAGCTCCACC

Supplemental Table 4. SLX4 siRNA

SLX4 siRNAs	TTTGGATGAAGATTCTGAGATCTG
	TTCCGTGGCTCCTTCTGCTGGTGG
	AAGAGTTCTGGAAATTCTCGGCC
XPF siRNAs	TCGAAATTACGCATATCC
	TGTATAGCAAGCATGGTAG
	AAGTCACCAACACAAGTATCC
MUS81 siRNAs	TTCTGAAATACGAAGCGCG
	AGAGGGTTGGAGAGGTCTG
	TTAGGATTCAAGGTGCTCCC

Supplemental Table 5. RT-qPCR primers

SLX4 qPCR	GCTGAAGAAGGAAGTGGATAGG
	TCCTTCAGCTTCAGAACCATC
Actin qPCR	GCTACGAGCTGCCTGACG
	GGCTGGAAGAGTGCCTCA